REMARKS

Applicants note with appreciation the detailed response to their arguments of September 9, 1992. The remarks and declarations presented herein are in response to the clarifications of the Examiner's previous rejections.

Although entry of the Rule 132 declarations is not required, Applicants request that the Examiner exercise her discretion and enter the declarations. The declarations are not introducing new issues and merely support previously articulated reasoning by Applicants' attorneys. Applicants believe these declarations are timely, do not place undue burden on the Examiner, and are merely placing factual support for Applicants' arguments in proper context for presentation to the Board of Appeal.

STATUS OF THE CLAIMS:

Claims 1-22 are pending. The disclosure was objected to because Claim 11 was grammatically awkward. The rejection of Claims 1-22 as obvious was maintained. The amendments to claims 11 and 17 are not believed to introduce new matter. Claim 11 was amended to correct a grammatical problem. Claim 17 now expressly recites a polymerase chain reaction [PCR] amplification primer pair. Such primer pairs are found throughout the specification wherever PCR is described.

THE INVENTION:

This invention provides for a means of simultaneously amplifying and detecting amplified target nucleic acid. Simultaneous amplification and detection is made possible through the use of DNA binding agents which give a photodetectable signal when bound to duplexed nucleic acid. Through the use photodetectors, it is possible to measure the amplification of nucleic acid while the reaction is proceeding.

SECTION 112:

Claim 11 was objected to as grammatically awkward. The amendment to claim 11 introduces the word "wherein" and follows the suggestion of the Examiner. Applicants believe the §112 rejection is overcome in view of the amendment. Applicants acknowledge the Examiner's withdrawal of the previous bases of rejection under §112.

SECTION 103:

The Examiner has maintained his original rejection of claims 1-22, under 35 USC §103, as being unpatentable over Sutherland et al. in view of Mullis et al. Sutherland is cited as disclosing "methods for the use of fluorescent dyes including in particular ethidium bromide for measurement of polymerization of nucleic acids during PCR amplification...that these dyes can be provided directly in the PCR reaction and that the dyes have a greater fluorescence when bound to double stranded DNA than when either bound by single stranded DNA or unbound." Mullis is cited as disclosing PCR.

Applicants respectfully request clarification. If Sutherland actually disclosed PCR, it would be an anticipatory reference over the original claims. The Examiner does not cite it as anticipatory and applicants wonder if she tacitly acknowledges that the Sutherland work merely uses PCR reagents but does not describe the multi-step procedure of PCR. Applicants previously explained that PCR requires two different primers to effect DNA amplification and Sutherland clearly uses a single primer to measure DNA elongation rates rather than rates of amplification for target DNA.

For purposes of appeal, applicants expressly rely on the various bases for traversing the *prima facie* case of obviousness set forth in their Response dated September 9, 1992. In brief, the Examiner has misinterpreted the Sutherland reference, there is no motivation to combine DNA binding agents into PCR

mixtures, there was literature teaching away from the use of fluorescent intercalating agents in PCR, and finally, applicants surprisingly demonstrate with ethidium bromide [EtBr] that there is no significant inhibition of PCR by intercalating agents at the recited concentrations.

The Examiner articulates motivation to combine Sutherland with Mullis stating that the method of detecting polymerization in an amplification reaction is suggested because Sutherland describes it as "very simple and minimizes sampling and handling errors".

Applicants find no fault with this assessment of Sutherland when applied to using DNA binding agents *to measure polymerase activity* in a single step polymerase reaction; but vigorously points out that the pending claims are *not* drawn to measuring polymerase activity. In contrast to Sutherland, applicants are measuring *amplification* of DNA. The Examiner is asked to reconsider this point in view of the discussion presented below.

1. Sutherland does not teach PCR - it is simply a misstatement by the patentees.

Applicants note with dismay that the Examiner on page 4 still believes that the Sutherland reference discloses fluorescent dyes with PCR. Two Rule 132 declarations are enclosed. The first declaration is by a co-inventor of the Sutherland patent. The second declaration is by the inventor of the subject application. Both declarants state with certainty that the language in Sutherland does not describe PCR and that the reference to PCR in Example 6 is a clear error.

Yes, the language of Example 6 states that PCR reagents and fluorescent dyes are being combined. And, at column 16, line 61, the patentees mistakenly state that the "polymerase chain reaction was then initiated". At another point in the text, column 17, lines 12-14 and 26, the patentees state that

they are using only one primer and were careful not to say "amplification" and expressly state that they initiate "reaction".

Applicants acknowledge that on its face the Sutherland reference refers to PCR; but the Examiner must go beyond the express words and read the entire reference. When taken out of context, recitation of PCR could be read as a suggestion; but, it is improper to dissect out parts of a reference and apply those parts outside of their original context. If it were otherwise, Examiner's would be free to reconstruct applicants' invention using hindsight.

In *Meyer*, the CCPA reversed the Board of Appeals' decision based on a misreading of ambiguous language. In *Meyer* the Appellant had claimed a method using alkali metal salts of chlorine and the Board rejected the claim as anticipated over a reference disclosing alkaline chlorine solutions. The Board focused on a single experiment describing an alkali (sodium) as an alkaline solution of bromide and ignored a second experiment using alkaline (calcium) chlorine. Satisfied that the prior art used alkaline in the generic sense and not a reference to alkali salts alone, the § 102 rejection was reversed and the applicants permitted to make a showing of advantageous properties.

In view of *Meyer*, applicants respectfully submit that the Examiner is required to read the entire passage of Sutherland referring to PCR. Therein it is clear beyond other interpretation that only one primer is being used and thus, PCR is not occurring and the experiments are merely measuring the quantity of polymerase and are not quantifying PCR amplification product. If the Examiner maintains that the Sutherland does suggest PCR, she is following in the mistaken footsteps of the *Meyer* board.

Having provided two Rule 132 Declarations explaining that Sutherland does not teach the use of intercalating agents in PCR, that the language relied upon by the Examiner is a mistake in fact by Sutherland *et al.* and that according

to *In re Meyer,* the mistake in fact should not constrain the Examiner in any way, the basis for the obviousness rejection is believed overcome.

2. In combination, Sutherland, when properly interpreted, cannot be modified by Mullis to achieve a working assay to measure polymerase.

The Examiner is respectfully reminded that a primary reference cannot be combined with a secondary reference if the combination would render the primary invention inoperable. The seminal case on this point is *In re Gordon*, 221 USPQ 1125 (CAFC, 1984). In *Gordon*, the invention was a blood filter and the prior art of French described a liquid strainer to remove water from oil. The prior art and invention were structurally similar except that the two machines were inverted relative to each other. The appellants successfully argued that if you inverted the prior art apparatus, it would not work to strain water from oil. The Federal Circuit agreed and stated at page 1127:

> The mere fact that the prior art could be so modified would not have made the modification obvious unless the prior art suggested the desirability of the modification...In deed, if the French apparatus were turned upside down, it would be rendered inoperable for its intended purpose...In effect French teaches away from the board's proposed modification.

Taking Sutherland as the primary reference and Mullis as the secondary reference, applicants submit that the combination is inoperable or would render the Sutherland assay so unreliable that it would be the equivalent of inoperable. The modification of Sutherland's assay by Mullis would involve substituting a single primer/single step extension reaction with the two primer/multi-step-thermal cycling reaction of PCR.

This two parameter modification would greatly complicate the underlying chemistry and kinetics of the Sutherland polymerase assay. The quantification of polymerase using the Sutherland assay relies on a consistent and

predictable rate of nucleotide extension provided by the polymerase enzyme. Although DNA binding agents have certain inhibitory effects, these effects are a controllable parameter in Sutherland's single step reaction system. A standard curve to accommodate incipient polymerase inhibition. To provide evidence of the impropriety of combining Sutherland with Mullis, applicants direct the Examiner's attention to the Rule 132 declaration of coinventor Russell Higuchi, Ph.D.

According to Dr. Higuchi, the use of PCR complicates the Sutherland assay in at least three distinct ways. Each effected parameter is altered in an unpredictable manner and each individually castes doubt upon the quantitative capabilities of the hypothetical PCR/Sutherland polymerase assay.

First, PCR uses repeated heating and cooling of the enzyme. These temperature changes will inevitably stress the polymerase enzyme in unpredictable ways. In addition to stressing the enzyme, the different temperatures will further complicate the reaction rate and reduce the accuracy and reproducibility of the polymerase quantification assay. Dr. Higuchi stresses at page 4 of his declaration that unlike the Sutherland assay, fluctuations in enzyme rates due to temperature will not negatively effect a PCR amplification where one measures product accumulation and not enzyme concentration based indirectly upon product accumulation.

Secondly, PCR uses two primers binding to different nucleic acid sequences. In addition, the polymerase extended nucleic acid sequences are not identical in PCR. For these reasons, it could be stated that PCR involves two separate extension reactions proceeding independent of each other. Because Sutherland relies upon predictable extension, introduction of a second simultaneous extension reaction will force the user to quantitate using an average between the two reactions. This two reaction average is inherently less accurate than a system designed for a single extension reaction. For the Examiner to properly combine Mullis and Sutherland references, she must explain why there is motivation to

complicate and reduce the accuracy of the quantitation method of Sutherland by introducing a second independent extension reaction.

Thirdly, the introduction of DNA binding agents will effect the melting of the nucleic acid duplexes in an unknown manner. This is irrelevant in an assay with a single reaction cycle such as Sutherland; but in a multi-cycle reaction efficiency of amplification will be effected and in turn the ability of the amplification process to measure polymerase activity will be effected in an unpredictable manner.

Dr. Higuchi has conducted experiments evaluating the ability of PCR to quantify polymerase in the presence of DNA binding agents. (See pages 4-6 of Dr. Higuchi's Declaration.) The results were quite poor as was expected. According to Dr. Higuchi, substitution of PCR for Sutherland's single step extension assay provides an assay that has virtually no practical utility. In Dr. Higuchi's work the PCR enzyme assay has a usable range of a single enzyme unit. In contrast, the Sutherland assay has a useful range from zero to thirty enzyme units (see Exhibit 2 of Dr. Higuchi's declaration). Finally, Dr. Higuchi explains that the inhibiting effect of DNA binding agents, while accountable in the Sutherland system, creates a dramatic negative impact on product accumulation in a PCR-based system such that one of skill would not be motivated to modify Sutherland's assay to include PCR. (See Exhibit 3 of Dr. Higuchi's declaration.)

Having provided scientifically sound reasons why Sutherland is not combinable with Mullis because the modifications of Mullis would render Sutherland inoperable, applicants ask that the rejection under §103 be reconsidered and withdrawn.

3. Mullis cannot be properly combined with Sutherland.

It is not clear whether the extant obviousness rejection relies upon Mullis as a primary or a secondary reference. The Examiner states on pages 5-6 of the Final Office Action dated November 24, 1992, that Mullis is only relied upon as

"evidence that the fluorescence produced would result from only the target DNA as during PCR only target DNA is produced even if the original solution contains a large amount of non-target DNAs". The significance of this rationale is not clear to the applicants. If the Examiner is *not* relying upon Mullis for recitation of PCR, applicants would ask for clarification in view of the failure of Sutherland to actually describe out PCR.

Despite the fact that the record is unclear as to whether Mullis is a primary or secondary reference, Applicants have also considered Mullis as a primary reference modified by the inclusion of ethidium bromide disclosed in Sutherland, et al. In Section 8 of his Rule 132 declaration, Dr. Higuchi explains why one of skill would not have been motivated to combine a binding agent of Sutherland with PCR of Mullis. In brief, the binding agents of Sutherland are known to inhibit polymerase activity.[1] Inhibition of polymerase activity is the antithesis of the purpose behind PCR, i.e., amplification of nucleic acid. For the Examiner to modify Mullis using Sutherland, she must find motivation in either reference for measuring amplification rates during PCR.

More specifically, DNA binding agents are known to inhibit polymerase activity. For this reason no ordinary artisan would have been motivated to modify the PCR of Mullis with a binding agent of Sutherland. Because Mullis is teaching amplification of nucleic acid, the only purpose of the adding DNA binding agents to PCR would be to measure amplification product.

Having explained that the only reason to add DNA binding agents to a PCR would be to quantify amplification product, one needs to evaluate whether the prior art references would motivate one to add such agents to a PCR assay. The answer according to Dr. Higuchi is a clear no. Prior to this invention, quantitation

---

[1]Applicants have attached as Exhibit 1, Kaledin, *et al.* 1981. Kaledin describes the inhibition of *Thermus flavus* DNA polymerase by various DNA binding agents including ethidium bromide. Applicants ask the Examiner to consider this reference in addition to the previously cited references disclosing polymerase inhibition by DNA binding agents.

of PCR product was carried out at the end of a select thermal cycle. It would be counter productive and counter intuitive to measure end product by adding a polymerase inhibitor which would have unknown inhibitory effects on the quantity of amplification product.

To illustrate the dramatic effect of slight inhibition of polymerase activity on the quantity of amplification product, Dr. Higuchi has attached a graphic representation of the impact of reduced amplification efficiency upon the percent of final product produced. The graphic representation are exhibits 3 and 4 of his declaration. The Examiner should note that the average number of thermal cycles for PCR is between 25 and 40. At thirty-seven cycles and 100 target copies, the decrease in final product at a 5% inhibition is over 50%. With a 10% inhibition, the amount of PCR product is decreased by 90% from the amount expected in the absence of inhibition.

In contrast, the subject invention was based upon the discovery that the inhibition of amplification at the recited concentrations was surprisingly low and that the outcome of the amplification products was not negatively affected by the DNA binding agents. This discovery gave rise to the invention of using the DNA binding agents to monitor amplification rates in commercial PCR. Commercial applications demand highly reproducible and consistent results. Such results were not predictable nor expected from the combination of Mullis and Sutherland.

In view of the amendments, applicants submit that the use of DNA binding agents in amplification reactions such as PCR at levels which do not significantly inhibit amplification is a surprising as well as non-obvious invention. This is apparent regardless of whether one considers Mullis to be a primary or a secondary reference. The combination of references fails to set forth a proper *prima facie* case of obviousness.

Finally, Applicants direct the Examiner's attention to Section 9 of Dr. Higuchi's declaration. Therein, he explains that adding DNA binding agents to a

PCR amplification prior to thermal cycling has certain advantages in contrast to adding the agents after thermal cycling. There was a surprisingly low inhibition at useful concentrations of binding agents. The surprising reproducibility of the reactions in the presence of ethidium bromide allows for "real-time" monitoring of the accumulation of PCR product. Exhibit 5 of Dr. Higuchi's declaration demonstrates that real-time continuous monitoring of a PCR permits one to calculate the concentration of target present in an original sample. The mathematical relationship is described in Exhibit 6 of Dr. Higuchi's declaration.

The significant advantage of real-time monitoring to measure the quantity of target in an original sample is the avoidance of the plateau effect. When PCRs are carried out beyond a given thermal cycle, the accumulation of PCR product plateaus. This is due to several factors including enzyme inactivation and lowering concentrations of primers and nucleotides triphosphates. The level and thermal cycle at which plateauing may occur is not necessarily a factor of the original concentration of target. For this reason, measuring accumulated product after thermal cycling is not as effective as real time continuous monitoring.

Dr. Higuchi refers to his invention as increasing the dynamic range or concentrations of target DNA for which PCR can be effectively used. Dr. Higuchi states that the dynamic range spans 6 orders of magnitude. The broad range allows for criminal or forensic applications of PCR where original levels of target are not in the control of the users. Other advantages of an increased dynamic range are where vast numbers of different samples are being analyzed or where one desires to monitor viral responses to drug therapy and the starting concentration of viral DNA may vary widely in a given sample.

Other advantages include avoidance of contamination of a PCR mixture because continuous monitoring can be achieved in a closed container. Lastly, the invention permits one to follow the progress of a given amplification. This has great importance to scientists developing reliable PCR test kits for

industrial and medical users. By monitoring the efficiency of various reactants such as primers or buffers under different conditions, one can better assess the reliability and reproducibility of a given PCR test kit product.

Applicants request reconsideration of the rejection of claims 1-22 as obvious. As explained above, the Examiner's interpretation of Sutherland is based on a literal interpretation of a sentence taken out of context and which is unsupported by a reading of the entire passage. Moreover, even if Sutherland was properly interpreted, the Examiner's *prima facie* case of obviousness would fail. There is lack of motivation to combine the references and there are references suggesting away from the use of DNA binding agents for amplification reactions. Finally applicants have described surprisingly advantageous results which permit quantifications of PCRs not previously possible.

Applicants will now address the remaining rejections, addressing each points in the order presented by the Examiner. We first address the rejection of dependent claim 10 in view of Sutherland and Mullis.

Claim 10 is dependent upon claim 1 and further recites that the amplified target is quantitated during the amplification rather than at the end of the amplification process. The Examiner argues that one of skill "would have known that one could quantitate the amount of target DNA ... by a simple comparison to a standard curve". Applicants would respectfully point out that quantitation is not always a simple matter of constructing a standard curve. In many cases the data points are so irreproducible that calculation of a standard curve is not possible.

Irreproducibility is the issue and modifying Sutherland by using PCR introduces new parameters which intuitively degrade the reproducibility of the Sutherland assay. This fact has been stated above and is evidenced by the Declaration of Dr. Higuchi. In Sutherland, the investigators are measuring the amount of polymerase by detecting the increase in double stranded DNA through the incorporation of the binding agents. Once the double strand is formed and the

agents interlaced within, the DNA can be inert. It does not need to perform again. The relative increases in fluorescence will correlate with the amount of the polymerase. The effect of these agents upon efficiency of the polymerase is of no importance to Sutherland because standard curves are produced which correct for the inhibition. In contrast, the pending claims, recite an invention where one is quantitatively measuring amplification product while it is amplifying. When one is attempting to quantify the amount of target DNA in sample, the efficiency of polymerase activity needs to taken into account. As explained in the previous Response and in Dr. Higuchi's Rule 132 Declaration, relatively small effects of polymerase efficiency have a dramatic impact on the quantity of amount of amplified target.

In summary, the applicants submit that the Sutherland method of using DNA binding agents to measure the amount of polymerase in a given sample using a single polymerase extension does not render obvious the subsequent use of those agents in PCR.

Claims 17-22 are directed to kits for carrying out PCR. The Examiner rejects claims 17-22 as obvious in view of Sutherland and Mullis for their respective teachings as recited earlier. PCR kits must have two amplification primers and are thus distinguished from the assays of Sutherland. Applicants rely on the arguments presented for claims 1-22 to overcome the extant obviousness rejection.

Claims 12-16 are dependant upon claim 11 and are directed to the use of optical fibers to measure the fluorescence increases arising from carrying out PCR in the presence of an intercalating agent. Applicants rely on the arguments presented for claims 1-22 to overcome the extant obviousness rejection.

Finally, the Examiner addresses the art submitted as teaching away. This art taught the inhibiting effects of DNA binding agents upon polymerase

activity. The Examiner dismissed their teaching based upon the fact that Sutherland taught to the contrary and was later in time. Having explained that Sutherland does not teach PCR, but only mistakenly recited PCR, applicants submit that the art is valid for teaching that polymerase activity is inhibited by DNA binding agents and should be considered for what it teaches. The Examiner will recall that this art was submitted for its suggesting away from the use of DNA binding agents in PCR. Applicant has previously explained and herein stated that the inhibitory effects of these agents upon polymerase activity would not preclude their use in the invention of Sutherland which is directed to measuring the amount of polymerase in a solution. This is in direct contrast to their addition to a PCR reaction.

Applicants believe that all the extant rejections have been fully addressed and overcome by amendment and argument.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned attorney.

Respectfully submitted,

Kenneth A. Weber
Reg. No. 31,677

TOWNSEND and TOWNSEND KHOURIE and CREW
One Market Plaza
Steuart Street Tower, 20th Floor
San Francisco, California 94105
Telephone: (415) 543-9600